JOUR UMRANIYE PEDIATR
2026;6(2):66–73 doi: 10.14744/upd.2026.07269

ORIGINAL RESEARCH
Pediatric Intensive Care

Respiratory viral coinfections and clinical outcomes in critically ill children with acute lower respiratory tract infections

Ebru Güney Şahin, Burhan Eloğlu, Kübra Boydağ Güvenç, Furkan Tosun, Cansu Durak, Fatih Varol

Department of Pediatric Intensive Care, University of Health Sciences, Sancaktepe Sehit Prof. Dr. Ilhan Varank Training and Research Hospital, Istanbul, Türkiye

ABSTRACT

Objective: To characterize the distribution of respiratory viruses, determine the frequency of multiple viral detection, and evaluate the association between viral detection patterns and clinical outcomes in children admitted to a pediatric intensive care unit (PICU) with acute lower respiratory tract infections (LRTIs).

Material and Methods: This retrospective observational cohort study included patients admitted to the PICU with LRTIs between September 2022 and December 2024 who underwent multiplex respiratory polymerase chain reaction (PCR) testing using nasopharyngeal specimens. Patients were evaluated according to respiratory panel positivity and viral burden. Demographic characteristics, laboratory findings, respiratory support requirements, length of stay, and mortality were compared.

Results: A total of 183 patients were included, with at least one virus detected in 126 (68.9%). Among virus-positive patients, 73 (57.9%) had single viral detection, and 53 (42.0%) had multiple viral detection. The most common viruses among patients with single viral detection were rhinovirus/enterovirus (17.8%), human bocavirus (15.1%), and respiratory syncytial virus (RSV) (15.1%); among those with multiple viral detection, they were rhinovirus/enterovirus (39.6%), RSV (37.7%), and human bocavirus (35.8%). Invasive mechanical ventilation was more frequent in patients with multiple viral detection than in those with single viral detection (52.8% vs. 34.2%, p=0.037), whereas hospital length of stay, PICU length of stay, and mortality did not differ significantly. Although PICU length of stay differed overall across the four viral-burden groups (p=0.029), no post hoc pairwise comparison remained significant after Bonferroni adjustment.

Conclusion: Multiple viral detection was common among virus-positive children with LRTIs and was associated with a higher frequency of invasive mechanical ventilation. No significant differences were observed in mortality or length of stay. Although PICU length of stay differed overall across viral-burden groups, pairwise comparisons were not significant; therefore, this finding should be considered exploratory. Larger prospective studies are needed to confirm these findings.

Keywords: Bronchiolitis; multiplex PCR; pediatric intensive care unit; pneumonia; respiratory syncytial virus; viral coinfection.

Cite this article as: Güney Şahin E, Eloğlu B, Boydağ Güvenç K, Tosun F, Durak C, Varol F. Respiratory viral coinfections and clinical outcomes in critically ill children with acute lower respiratory tract infections. Jour Umraniye Pediatr 2026;6(2):66–73.

Received (Başvuru): 28.07.2026 **Revised (Revizyon):** 25.08.2026 **Accepted (Kabul):** 01.09.2026 **Online (Online yayınlanma):** 11.09.2026

Correspondence (İletişim): Dr. Ebru Güney Şahin. Sağlık Bilimleri Üniversitesi, Sancaktepe Şehit Prof. Dr. İlhan Varank Eğitim ve Araştırma Hastanesi, Çocuk Yoğun Bakım Klinigi, İstanbul, Türkiye.
Phone (Tel): +90 530 403 81 70 **e-mail (e-posta):** ebruguneysahin@hotmail.com

Akut alt solunum yolu enfeksiyonu nedeniyle yoğun bakımda izlenen kritik hasta çocuklarda solunum yolu viral koenfeksiyonları ve klinik sonuçları

ÖZET

Amaç: Bu çalışmanın amacı, akut alt solunum yolu enfeksiyonu (ASYE) nedeniyle çocuk yoğun bakım ünitesine (ÇYBÜ) yatırılan çocuklarda solunum yolu virüslerinin dağılımını belirlemek, çoklu viral saptanma sıklığını değerlendirmek ve viral saptanma paternleri ile klinik sonuçlar arasındaki ilişkiyi incelemektir.

Gereç ve Yöntemler: Bu retrospektif gözlemsel kohort çalışmasına, Eylül 2022 ile Aralık 2024 tarihleri arasında akut alt solunum yolu enfeksiyonu tanısıyla ÇYBÜ'ye yatırılan ve nazofaringeal örneklerden multipleks solunum yolu polimeraz zincir reaksiyonu (PCR) testi yapılan hastalar dahil edildi. Hastalar, solunum yolu paneli pozitifliği ve viral yük açısından değerlendirildi. Demografik özellikler, laboratuvar bulguları, solunum desteği gereksinimleri, yatış süreleri ve mortalite karşılaştırıldı.

Bulgular: Toplam 183 hasta çalışmaya dahil edildi ve 126 hastada (%68,9) en az bir solunum yolu virüsü saptandı. Virüs pozitif hastaların 73'ünde (%57,9) tek, 53'ünde (%42,0) ise birden fazla virüs saptandı. Tek virüs saptanan hastalarda en sık rinovirüs/enterovirüs (%17,8), human bocavirus (%15,1) ve respiratuvar sinsityal virüs (RSV) (%15,1); birden fazla virüs saptanan hastalarda ise rinovirüs/enterovirüs (%39,6), RSV (%37,7) ve human bocavirus (%35,8) belirlendi. İnvaziv mekanik ventilasyon gereksinimi, birden fazla virüs saptanan hastalarda tek virüs saptananlara göre daha sıktı (%52,8'e karşı %34,2; p=0,037). Buna karşılık, hastanede ve ÇYBÜ'de yatış süreleri ile mortalite açısından gruplar arasında anlamlı fark saptanmadı. Saptanan virüs sayısına göre oluşturulan dört grup arasında ÇYBÜ yatış süresi genel analizde farklılık gösterdi (p=0,029); ancak Bonferroni düzeltmesi sonrasında ikili karşılaştırmaların hiçbiri istatistiksel olarak anlamlı değildi.

Tartışma: Solunum yolu virüsü saptanan ASYE'li çocuklarda birden fazla virüs saptanması sık görülmüş ve daha yüksek invaziv mekanik ventilasyon gereksinimi ile ilişkili bulunmuştur. Mortalite ve yatış süreleri açısından anlamlı fark saptanmamıştır. Saptanan virüs sayısına göre ÇYBÜ yatış süresi genel analizde farklılık gösterse de ikili karşılaştırmalar anlamlı bulunmadığından, bu sonuç keşifsel niteliktedir. Bulguların doğrulanması için daha geniş, prospektif çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Bronşiolit; çocuk yoğun bakım ünitesi; multipleks PCR; pnömoni; respiratuvar sinsityal virüs; viral koenfeksiyon.

ORCID ID

EGŞ: 0000-0002-7532-7724; BE: 0009-0002-6150-0626; KBG: 0000-0003-3881-6980; FT: 0009-0008-5919-431X; CD: 0000-0001-6309-8859; FV: 0000-0002-2424-6887

Sağlık Bilimleri Üniversitesi, Sancaktepe Şehit Prof. Dr. İlhan Varank Eğitim Ve Araştırma Hastanesi, Çocuk Yoğun Bakım Bilim Dalı, İstanbul, Türkiye

INTRODUCTION

Among children, lower respiratory tract infections (LRTIs) are among the most frequent reasons for hospital admission and remain an important cause of morbidity and mortality, especially in early childhood. The majority of these infections are viral in origin, with respiratory syncytial virus (RSV), rhinovirus, influenza and parainfluenza viruses, human metapneumovirus, and human bocavirus among the pathogens most often implicated (1, 2).

Multiplex PCR platforms now allow several respiratory pathogens to be identified from a single nasopharyngeal sample, and their growing clinical use has markedly increased detection sensitivity relative to older single-target assays. As a result, coinfection with more than one respiratory virus is being reported more often among children hospitalized with LRTIs, with published detection rates ranging from approximately 10%-40% and occasionally exceeding this range in selected populations (3, 4). However, it remains uncertain whether the detection of multiple viruses represents true concurrent infection or reflects prolonged viral shedding or asymptomatic carriage.

Findings on this point are inconsistent: some individual studies link coinfection with longer hospital stays and more severe disease (4). Yet, pooled data from systematic reviews and meta-analyses have generally failed to show consistent differences between children with single versus multiple viral detections in terms of intensive care admission, the need for mechanical ventilation, length of stay, or mortality (3). Because of this inconsistency, research interest has shifted from simply asking whether multiple viral detection is present to examining the number and patterns of viruses detected, on the premise that these features may better characterize the heterogeneity of clinical outcomes. Previous studies have suggested that the number of detected viruses may be associated with disease severity and resource utilization, although the clinical significance of multiple viral detection remains uncertain (5, 6).

The present study aimed to characterize the distribution of respiratory viruses, determine the frequency of multiple viral detection, compare the clinical characteristics and outcomes of children with single and multiple viral detection, and explore the association between viral burden and clinical outcomes among patients admitted to a pediatric intensive care unit with acute LRTIs.

MATERIAL AND METHODS

Study Design and Patient Selection

This retrospective observational cohort study was conducted in a PICU between September 2022 and December 2024. During the study period, 782 patients were admitted to the PICU with LRTIs. Respiratory multiplex PCR testing was not routinely performed in all patients with LRTIs; testing was requested at the discretion of the treating physician based on clinical suspicion and was also influenced by test availability. Consequently, only patients who underwent respiratory multiplex PCR testing were eligible for the present study, resulting in a final study cohort of 183 patients. Because patients who were not tested were not included in the analytic cohort, the study population represents a selected subset of PICU patients with LRTIs rather than the entire LRTI population admitted during the study period.

The study was approved by the Scientific Research Ethics Committee of Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital (Approval No.: 2026/13; approval date: 14.01.2026). All procedures were conducted in accordance with the principles of the Declaration of Helsinki. Owing to the retrospective nature of the study and the use of anonymized data, the requirement for written informed consent was waived by the ethics committee.

Patients admitted to the PICU with a diagnosis of LRTI who underwent multiplex respiratory PCR testing using nasopharyngeal specimens at admission or within the first 48 hours of hospitalization were eligible for inclusion. Patients with incomplete clinical records or unavailable PCR results were excluded from the study.

Definitions

A diagnosis of pneumonia required compatible clinical signs supported by radiographic evidence of pulmonary infiltration (7). Bronchiolitis was diagnosed clinically according to current pediatric guideline criteria, particularly in children younger than 2 years of age presenting with wheezing, tachypnea, and increased respiratory effort following a preceding viral upper respiratory illness (8).

Multiplex Respiratory PCR Analysis

Nasopharyngeal swab specimens were obtained as part of routine clinical practice and analyzed using a multiplex respiratory polymerase chain reaction (PCR) assay. Respiratory pathogens were detected using the Respiratory Pathogens Kit (24) (Laborant, Ref No.: LRP-01-24, AMT Medical Laboratory Equipment Industry and Trade Ltd. Co., Istanbul, Türkiye). The assay included rhinovirus/enterovirus, respiratory syncytial virus (RSV), influenza A and B viruses, parainfluenza virus types 1–4, adenovirus, human metapneumovirus, seasonal coronaviruses, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), human bocavirus, and several bacterial and atypical bacterial pathogens.

For the purposes of the present study, analyses were restricted to viral pathogens. Although the multiplex panel also included bacterial and atypical bacterial targets, these organisms were excluded from multiple viral detection analyses because the primary objective was to evaluate the distribution and clinical significance of multiple respiratory viral detection and viral burden. Inclusion of bacterial targets would have introduced a biologically distinct category of mixed viral–bacterial infections requiring separate analysis.

Data Collection

Demographic and clinical data were extracted from electronic medical records, including age, sex, history of prematurity, presence of comorbidities, Pediatric Risk of Mortality III (PRISM III) score, admission diagnosis, respiratory PCR results, and identified viruses (9).

Laboratory variables included C-reactive protein (CRP), procalcitonin (PCT), white blood cell count, neutrophil count, lymphocyte count, pH, partial pressure of carbon dioxide ($PaCO_2$), and lactate levels.

Clinical outcome measures included the requirement for high-flow nasal cannula (HFNC), non-invasive ventilation (NIV), and invasive mechanical ventilation (IMV), together with the duration of each respiratory support modality. Pediatric intensive care unit length of stay, total hospital length of stay, and mortality were also recorded. In addition, respiratory panel positivity, the distribution of detected viruses, viral burden, and seasonal patterns were evaluated.

Study Groups

Among PCR-positive patients, virus distribution was characterized according to the number of viral targets detected. Patients with one detected virus were classified as having single viral detection, whereas those with two or more detected viruses were classified as having multiple viral detection. For descriptive purposes, the number of viruses detected in each patient (one, two, three, or four) was defined as viral burden. Because multiplex PCR detects viral nucleic acid and does not establish active viral replication, the terms “single viral detection” and “multiple viral detection” were preferred over “single viral infection” and “viral coinfection” when referring specifically to PCR findings. Viral-burden analyses were considered exploratory.

Statistical Analysis

All statistical analyses were performed using SPSS (IBM SPSS Statistics for Windows, Version 22.0; IBM Corp., Armonk, NY, USA). Normality of continuous variables was assessed using the Kolmogorov–Smirnov test together with visual inspection of histograms. Continuous variables were reported as median (IQR), and categorical variables were reported as counts and percentages. Because the continuous variables included in Tables 1 and 2 were non-normally distributed, comparisons between two independent groups were performed using the Mann–Whitney U test. Categorical variables were compared using Pearson’s chi-square test or Fisher’s exact test, as

Table 1. Comparison of demographic, clinical, and outcome characteristics according to respiratory panel positivity

	Negative (n=57)	Positive (n=126)	p
Age, months, median (IQR)	18.5 (4-52.75)	10 (2-29.5)	0.086
Male sex, n (%)	32 (56.1)	66 (52.4)	0.637
Comorbidity, n (%)	13 (22.8)	20 (15.9)	0.259
PRISM III score, median (IQR)	5 (0-9.25)	3 (0-6)	0.099
Hospital LOS, days, median (IQR)	8 (7-18)	9 (7-15)	0.861
PICU LOS, days, median (IQR)	8 (5.75-14.25)	9 (5-14)	0.762
Mortality, n (%)	3 (5.3)	3 (2.4)	0.377
IMV, n (%)	24 (42.1)	53 (42.1)	0.996
NIV, n (%)	30 (52.6)	79 (62.7)	0.199
HFNC, n (%)	30 (52.6)	71 (56.3)	0.640
Radiographic infiltrate, n (%)	41 (71.9)	99 (78.6)	0.326

Data are presented as median (IQR) or n (%). Percentages were calculated within each study group. Continuous variables were compared using the Mann–Whitney U test. Fisher's exact test was used for mortality; Pearson's chi-square test was used for all other categorical variables. PICU: Pediatric intensive care unit; LOS: Length of stay; IMV: Invasive mechanical ventilation; NIV: Non-invasive ventilation; HFNC: High-flow nasal cannula.

Table 2. Comparison of demographic, clinical, and outcome characteristics according to single and multiple viral detection

	Single viral detection (n=73)	Multiple viral detection (n=53)	p
Age, months, median (IQR)	12 (3-33)	9 (2-21.5)	0.143
Male sex, n (%)	41 (56.2)	25 (47.2)	0.318
Prematurity, n (%)	17 (23.3)	16 (30.2)	0.384
Comorbidity, n (%)	12 (16.4)	8 (15.1)	0.839
PRISM III score, median (IQR)	3 (0-6)	3 (0-7)	0.663
Hospital LOS, days, median (IQR)	9 (7-14)	10 (6-17)	0.789
PICU LOS days, median (IQR)	9 (6-13)	9 (5-15.5)	0.768
Mortality, n (%)	2 (2.7)	1(1.9)	1.000
IMV, n (%)	25 (34.2)	28 (52.8)	**0.037**
NIV, n (%)	43 (58.9)	36 (67.9)	0.301
HFNC, n (%)	45 (61.6)	26 (49.1)	0.160
Radiographic infiltrate, n (%)	56 (76.7)	43 (81.1)	0.550

Data are presented as median (IQR) or n (%). Continuous variables were compared using the Mann–Whitney U test. Fisher's exact test was used for mortality; Pearson's chi-square test was used for all other categorical variables. PICU: Pediatric intensive care unit; LOS: Length of stay; IMV: Invasive mechanical ventilation; NIV: Non-invasive ventilation; HFNC: High-flow nasal cannula.

appropriate. Comparisons across the four viral-burden groups were performed using the Kruskal–Wallis test. When the overall test was statistically significant, pairwise post hoc comparisons with Bonferroni adjustment for multiple testing were performed. A two-sided p value<0.05 was considered statistically significant.

RESULTS

Among the 183 patients with LRTIs who underwent respiratory multiplex PCR testing and were included in the study, at least one virus was detected in 126 (68.9%), whereas 57 (31.1%) had no viral target detected. Among virus-positive patients, 73 (57.9%) had single viral detection, and 53 (42.0%) had multiple viral detection.

No significant differences were observed between respiratory panel-positive and respiratory panel-negative patients with respect to age, sex, presence of comorbidities, PRISM III score, hospital length of stay, PICU length of stay, mortality, or respiratory support requirements (Table 1).

Respiratory panel positivity varied significantly according to the month of admission (p=0.001). The highest positivity rates were observed in December (91.7%), April (85.7%), and January (84.6%), whereas no positive cases were identified in July or August. Seasonal positivity rates were 80.6% in winter, 74.1% in

Table 3. Distribution of detected viruses and viral burden among patients with positive respiratory panels (n=126)

Variables	n	%
Single viral detection	73	57.9
Rhinovirus/Enterovirus	13	17.8
Human bocavirus	11	15.1
RSV	11	15.1
Adenovirus	7	9.6
Influenza B	6	8.2
Parainfluenza virus	6	8.2
Influenza A	5	6.8
Seasonal coronavirus	5	6.8
SARS-CoV-2	5	6.8
Human metapneumovirus	4	5.5
Number of detected viruses (n=126)		
1 virus	73	57.9
2 viruses	35	27.8
3 viruses	15	11.9
4 viruses	3	2.4
Most frequently detected viruses among patients with multiple viral detection (n=53)		
Rhinovirus/Enterovirus	21	39.6
RSV	20	37.7
Human bocavirus	19	35.8
Most common multiple viral detection combinations (n=53)		
RSV + Rhinovirus/Enterovirus	10	18.9
Human bocavirus + RSV	8	15.1
Human bocavirus + Rhinovirus/Enterovirus	6	11.3

Data are presented as n (%). Percentages for individual viruses in the single viral detection group were calculated among patients with single viral detection (n=73). Percentages for the number of detected viruses were calculated among all respiratory panel-positive patients (n=126). Percentages for viruses and viral combinations in the multiple viral detection group were calculated among patients with multiple viral detection (n=53). RSV: Respiratory syncytial virus; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

spring, 47.8% in summer, and 47.1% in autumn. No significant differences in positivity rates were observed across the study years (p=0.395).

Among patients with single viral detection, the most frequently identified viruses were rhinovirus/enterovirus (17.8%), human bocavirus (15.1%), RSV (15.1%), and adenovirus (9.6%). Influenza B and parainfluenza viruses were each detected in 8.2% of cases, whereas influenza A, seasonal coronaviruses, and SARS-CoV-2 were each identified in 6.8% of cases. Human metapneumovirus was the least frequently detected virus among patients with single viral detection, accounting for 5.5% of cases (Table 3).

Comparison of patients with single and multiple viral detection revealed no significant differences in age, sex, prematurity, comorbidity status, PRISM III score, hospital length of stay, PICU length of stay, mortality, NIV requirement, HFNC requirement, or radiographic infiltrates. However, invasive mechanical ventilation was more frequent among patients with multiple viral detection than among those with single viral detection (52.8% vs. 34.2%, p=0.037) (Table 2).

The frequency of multiple viral detection did not differ significantly across seasons (p=0.472) or study years (p=0.396).

Among laboratory variables, lactate levels were higher in patients with multiple viral detection than in those with single viral detection (1.6 [1.17–2.27] mmol/L vs. 1.35 [0.91–1.76] mmol/L, p=0.028). HFNC duration was shorter in patients with multiple viral detection than in those with single viral detection [0 (0–3) days vs. 2 (0–4) days, p=0.043]. No statistically significant differences were observed in the other laboratory parameters or in IMV and NIV durations (Appendix 1).

When patients were stratified according to the number of detected viruses, PICU length of stay differed significantly across the four viral-burden groups in the overall Kruskal–Wallis analysis (H=9.018, df=3, p=0.029) (Appendix 2). Median PICU length of stay was 9 (6–13) days in patients with one detected virus, 7 (5–11) days in those with two viruses, 13 (7.5–18.25) days in those with three viruses, and 50 (11–NA) days in those with four viruses. However, none of the post hoc pairwise comparisons remained statistically significant after Bonferroni adjustment for multiple comparisons (all adjusted p≥0.136) (Appendix 3). These findings should be interpreted cautiously, particularly because the higher viral-burden subgroups were small and the four-virus group included only three patients.

Among patients with multiple viral detection, the most frequently detected viruses were rhinovirus/enterovirus (n=21, 39.6%), RSV (n=20, 37.7%), and human bocavirus (n=19, 35.8%). The most common multiple viral detection combinations were RSV–rhinovirus/enterovirus (n=10, 18.9%), human bocavirus–RSV (n=8, 15.1%), and human bocavirus–rhinovirus/enterovirus (n=6, 11.3%) (Table 3).

DISCUSSION

Among the selected cohort of PICU patients with LRTIs who underwent respiratory multiplex PCR testing, at least one respiratory virus was detected in 68.9%, and 42.0% of virus-positive patients had multiple viral detections. These proportions should be interpreted within the tested cohort and should not be considered estimates of viral positivity or multiple viral detection among all children admitted to the PICU with LRTIs. Multiple viral detection was associated with a higher frequency of invasive mechanical ventilation, whereas no statistically significant differences were detected in hospital length of stay, PICU length of stay, mortality, NIV requirement, or HFNC requirement.

As multiplex PCR testing has become routine, detecting more than one respiratory virus in a single pediatric sample is now

common, yet the clinical significance of this finding remains debated. In a systematic review including more than 17,000 children, Scotta and colleagues could not demonstrate any association between coinfection and hospital length of stay, PICU admission, ventilation requirement, or mortality (3). Asner et al. (10) reached a similar conclusion, reporting comparable hospitalization duration and mortality regardless of whether children carried one virus or several. Our findings were partly consistent with these reports, as multiple viral detection was not associated with differences in length of stay or mortality. However, IMV was more frequent among patients with multiple viral detection in our cohort. This finding suggests a possible association between multiple viral detection and the need for invasive respiratory support, although the retrospective design precludes causal inference, and residual confounding cannot be excluded.

Although HFNC duration was shorter in patients with multiple viral detection, this finding should be interpreted cautiously. Given the higher frequency of IMV in this group, differences in respiratory support trajectories may have influenced HFNC exposure; however, the timing and sequence of transitions between respiratory support modalities were not evaluated in the present study.

One important limitation in interpreting multiplex PCR results is that the detection of multiple viruses does not necessarily represent simultaneous active infections. Rhinovirus and human bocavirus, in particular, are known to persist in the airway well after symptoms resolve and can even be identified in children without respiratory complaints. Some cases we classified as multiple viral detection may therefore reflect one active infection alongside residual shedding of a second virus rather than genuine concurrent disease. This uncertainty complicates the interpretation of the observed associations between multiple viral detection and clinical outcomes. Accordingly, our findings should be interpreted as associations between viral detection patterns and outcomes rather than as evidence of causality (3, 4).

Among patients with single viral detection, rhinovirus/enterovirus, human bocavirus, and RSV were the most frequently detected viruses. Earlier work, especially in bronchiolitis cohorts, has repeatedly identified RSV as the leading respiratory pathogen, with rhinovirus close behind in frequency (2, 5). The relatively high frequency of human bocavirus observed in our cohort is consistent with earlier reports describing its common detection among hospitalized children (2). However, the role of human bocavirus in LRTIs remains incompletely understood. Because human bocavirus is frequently detected alongside other respiratory viruses and may exhibit prolonged viral shedding, PCR positivity does not necessarily indicate active disease (1, 2). Therefore, the clinical significance of human bocavirus detection should be interpreted in conjunction with the overall clinical and laboratory context.

Notably, rhinovirus/enterovirus, RSV, and human bocavirus were also the most commonly identified viruses among patients with multiple viral detection. This finding indicates that these viruses not only represent commonly detected viruses in patients with single viral detection but also contribute substantially to viral detection patterns in children with LRTIs. Consistent with this observation, the most frequent viral combinations in our cohort were RSV–rhinovirus/enterovirus, human bocavirus–RSV, and human bocavirus–rhinovirus/enterovirus. Similar coinfection patterns have been reported previously, with RSV–rhinovirus and RSV–human bocavirus among the most commonly described viral combinations in pediatric respiratory infections (2, 4). However, because rhinovirus and human bocavirus are also among the viruses most frequently associated with prolonged shedding, caution is warranted when interpreting the clinical significance of specific viral combinations or increasing viral burden.

Respiratory panel positivity showed clear seasonality, peaking in winter and spring—a pattern that mirrors the well-documented seasonal circulation of RSV and related viruses (1, 2). The frequency of multiple viral detection remained relatively stable across seasons. The mechanisms underlying this pattern could not be evaluated in the present study. Virus–virus interactions and differences in host susceptibility have been proposed as possible explanations, but these mechanisms remain hypothetical in the context of our data.

Patients with multiple viral detection also had modestly higher lactate levels than those with single viral detection; however, the clinical relevance of this isolated laboratory finding remains uncertain and may reflect residual confounding rather than a direct effect of multiple viral detection.

An exploratory analysis demonstrated an overall difference in PICU length of stay across viral-burden groups; however, none of the pairwise comparisons remained significant after Bonferroni adjustment, and the higher viral-burden groups were small. Previous studies have suggested that the number of detected viruses may be associated with clinical outcomes and resource utilization (5, 6). Bermúdez-Barrezueta et al. (6) reported progressively longer hospital stays with increasing numbers of detected viruses. However, their findings concerned hospital rather than PICU length of stay. Therefore, our finding should be considered exploratory rather than evidence of a dose–response or causal relationship.

Limitations

This study has several limitations. First, its single-center, retrospective design limits causal inference and generalizability. Of the 782 patients admitted to the PICU with LRTIs during the study period, only 183 underwent respiratory multiplex PCR testing and were included in the analysis. Testing was performed according to clinical judgment and test availability rather than through a systematic screening protocol. This may have preferentially selected patients with suspected viral disease or particular clinical presentations. Consequently, the observed 68.9% viral detection rate applies only to the tested cohort and cannot be extrapolated to the entire population of PICU patients with LRTIs. Because patients who were not

tested were not included in the analytic dataset, a systematic comparison of tested and untested patients was not possible, and residual selection bias cannot be excluded.

A second limitation is inherent to the technology itself: multiplex PCR identifies viral nucleic acid rather than active replication, so some positive results may reflect prolonged viral shedding, asymptomatic carriage, or concurrent active infection. These results should therefore be interpreted as descriptive associations between viral detection patterns and outcomes rather than as evidence of a causal relationship.

Third, all multiple viral detections were analyzed as a single group, although different viral combinations may differ biologically and clinically. The viral-burden subgroups with three or four detected viruses were also small, including only three patients with four detected viruses. Accordingly, findings from the viral-burden analysis should be considered exploratory and hypothesis-generating rather than confirmatory. In addition, the small number of deaths limited the statistical power to detect differences in mortality. More generally, the absence of statistically significant differences should not be interpreted as evidence of equivalence, particularly for infrequent outcomes and analyses involving small subgroups.

Finally, because quantitative viral load data were unavailable, the behavior of specific viral combinations could not be assessed, leaving the individual contribution of each virus within a multiple viral detection unresolved.

Notwithstanding these limitations, the present study provides real-world data on respiratory viral detection patterns and their associations with clinical outcomes in critically ill children with LRTIs.

CONCLUSION

Multiple respiratory viral detections were common among virus-positive children in this selected cohort of critically ill patients with LRTIs and were associated with a higher frequency of invasive mechanical ventilation. No statistically significant differences were detected in several other major clinical outcomes, including mortality and length of stay; however, the limited sample size and small number of outcome events preclude conclusions regarding equivalence between single and multiple viral detection. Although PICU length of stay differed across viral-burden groups in the overall analysis, none of the pairwise comparisons remained statistically significant after adjustment for multiple testing. Given the small number of patients with three or four detected viruses, this finding should be regarded as exploratory. Larger prospective studies using systematic respiratory testing are needed to clarify the clinical relevance of multiple viral detection, viral burden, and specific viral combinations.

Ethics Committee Approval: This study was approved by the Ethics Committee of University of Health Sciences, Sancaktepe Sehit Prof. Dr. Ilhan Varank Training and Research Hospital (Date: Jan 14, 2026, Decision No. 2026/13).

Informed Consent: The ethics committee has waived the requirement for written informed consent.

Conflict of Interest: The author declare that there is no conflict of interest.

Financial Disclosure: The authors declared that this study received no financial support.

Use of AI for Writing Assistance: The authors used ChatGPT (OpenAI) solely to improve the language, grammar, and readability of the manuscript. All scientific content, study design, data analysis, interpretation of the results, and final approval of the manuscript were performed by the authors, who take full responsibility for the content.

Authorship Contributions: Concept – EGŞ, CD; Design – FV, KBG; Supervision – FV; Data Collection and/or Processing – BE, FT, KBG; Analysis and/or Interpretation – EBŞ, CD; Literature Search – EGŞ, BE; Writing – EGŞ, CD; Critical Reviews – FV, CD.

Acknowledgments: The authors thank Prof. Dr. Halit Cam for his academic support and mentorship.

Peer-review: Externally peer-reviewed.

Etik Kurul Onayı: Bu çalışma, Sağlık Bilimleri Üniversitesi, Sancaktepe Şehit Prof Dr İlhan Varank Eğitim Ve Araştırma Hastanesi Etik Kurulu tarafından onaylanmıştır (Tarih: 14 Ocak 2026, Karar No: 2026/13).

Hasta Onamı: Etik kurulu, yazılı bilgilendirilmiş onam şartından feragat etmiştir.

Çıkar Çatışması: Yazar, herhangi bir çıkar çatışması bulunmadığını beyan eder.

Mali Destek: Yazarlar bu çalışma için mali destek almadıklarını beyan etmişlerdir.

Yazma Yardımı için Yapay Zeka Kullanımı: Yazarlar, ChatGPT'yi (OpenAI) yalnızca makalenin dilini, dilbilgisini ve okunabilirliğini iyileştirmek amacıyla kullanmışlardır. Tüm bilimsel içerik, çalışma tasarımı, veri analizi, sonuçların yorumlanması ve makalenin nihai onayı yazarlar tarafından gerçekleştirilmiştir; yazarlar, içeriğin tüm sorumluluğunu üstlenmektedir.

Yazarlık Katkıları: Fikir – EGŞ, CD; Tasarım – FV, KBG; Denetlemeler – FV; Veri toplanması ve/veya işleme – BE, FT, KBG; Analiz ve/veya yorumlama – EBŞ, CD; Literatür araştırması – EGŞ, BE; Yazım – EGŞ, CD; Eleştirel incelemeler – FV, CD.

Teşekkürler: Yazarlar, akademik desteği ve rehberliği için Prof. Dr. Halit Cam'a teşekkür ederler.

Hakemli inceleme: Harici olarak hakemli.

REFERENCES

1. Rhedin S, Lindstrand A, Hjelmgren A, Ryd-Rinder M, Öhrmalm L, Tolfvenstam T, et al. Respiratory viruses associated with community-acquired pneumonia in children: matched case-control study. Thorax 2015;70:847–53.
2. Kenmoe S, Kengne-Nde C, Ebogo-Belobo JT, Mbaga DS, Fatawou Modiyinji A, Njouom R. Systematic review and meta-analysis of the prevalence of common respiratory viruses in children < 2 years with bronchiolitis in the pre-COVID-19 pandemic era. PLoS One 2020;15:e0242302.
3. Scotta MC, Chakr VC, de Moura A, Becker RG, de Souza AP, Jones MH, et al. Respiratory viral coinfection and disease severity in children: A systematic review and meta-analysis. J Clin Virol 2016;80:45–56.
4. Calvo C, García-García ML, Blanco C, Vázquez MC, Frías ME, Pérez-Breña P, et al. Multiple simultaneous viral infections in infants with acute respiratory tract infections in Spain. J Clin Virol 2008;42:268–72.

5. Mansbach JM, Piedra PA, Teach SJ, Sullivan AF, Forgey T, Clark S, et al. Prospective multicenter study of viral etiology and hospital length of stay in children with severe bronchiolitis. Arch Pediatr Adolesc Med 2012;166:700–6.
6. Bermúdez-Barrezueta L, López-Casillas P, Rojo-Rello S, Sáez-García L, Marugán-Miguelsanz JM, Pino-Vázquez MA. Outcomes of viral coinfections in infants hospitalized for acute bronchiolitis. Virol J 2023;20:235.
7. St Peter SD, Ampofo K, Brogan T, Cabana MD, Espinosa C, Florin TA, et al. Clinical practice guideline by the infectious diseases society of America and the pediatric infectious diseases society: 2026 Guideline update on the management of community-acquired pneumonia in infants and children older than 3 months of age. Clin Infect Dis 2026:ciag186.
8. Ralston SL, Lieberthal AS, Meissner HC, Alverson BK, Baley JE, Gadomski AM, et al. Clinical practice guideline: the diagnosis, management, and prevention of bronchiolitis. Pediatrics 2014;134:e1474–502. Erratum in: Pediatrics 2015;136:782.
9. Pollack MM, Patel KM, Ruttimann UE. PRISM III: an updated Pediatric Risk of Mortality score. Crit Care Med 1996;24:743–52.
10. Asner SA, Science ME, Tran D, Smieja M, Merglen A, Mertz D. Clinical disease severity of respiratory viral co-infection versus single viral infection: a systematic review and meta-analysis. PLoS One 2014;9:e99392.

Appendix 1. Laboratory parameters and respiratory support durations according to single and multiple viral detection

Variables	Single viral detection (n=73)	Multiple viral detection (n=53)	p
IMV durations, days	0 (0-4)	0 (0-7)	0.152
NIV durations, days	2 (0-4)	2 (0-4)	0.504
HFNC duration, days	2 (0-4)	0 (0-3)	**0.043**
CRP, mg/L	11.7 (2.5-61.6)	16.4 (6.8-50.6)	0.364
Procalcitonin, ng/mL	0.26 (0.12-1.02)	0.3 (0.15-2.98)	0.250
White blood cell count, /µL	9190 (6280-14040)	10190 (6865-16145)	0.229
Neutrophil count, /µL	6000 (2980-10560)	6600 (3685-11720)	0.322
Lymphocyte count, /µL	2110 (1220-3150)	2300 (1415-3845)	0.296
pH	7.33 (7.26-7.39)	7.31 (7.25-7.37)	0.313
$PaCO_2$, mmHg	42.8 (35.7-54.0)	41.2 (37.8-52.6)	0.465
Lactate, mmol/L	1.35 (0.91-1.76)	1.6 (1.17-2.27)	**0.028**

Data are presented as median (IQR). Comparisons were performed using the Mann–Whitney U test. IMV: Invasive mechanical ventilation; NIV: Non-invasive ventilation; HFNC: High-flow nasal cannula; CRP: C-reactive protein; $PaCO_2$: Partial pressure of carbon dioxide; n: Number of patients.

Appendix 2. PICU length of stay according to viral burden

Number of detected virus	n	PICU LOS, median (IQR), days
1 virus	73	9 (6-13)
2 viruses	35	7 (5-11)
3 viruses	15	13 (7.5-18.25)
4 viruses	3	50 (11-NA) *
Overall p value		**0.029**

Overall Kruskal–Wallis test: H=9.018, df=3, p=0.029. *: IQR could not be fully calculated for the four-virus group because of the small sample size (n=3). PICU: Pediatric intensive care unit; LOS: Length of stay; IQR: Interquartile range; df: Degrees of freedom; NA: Not applicable; n: Number of patients.

Appendix 3. Bonferroni-adjusted pairwise comparisons of PICU length of stay across viral-burden groups

Pairwise comparison	Test statistic	Standard error	Standardized test statistic	Unadjusted p value	Bonferroni-adjusted p value
Two viruses vs. one virus	7.685	7.411	1.037	0.300	1.000
Two viruses vs. three viruses	-25.750	11.461	-2.247	0.025	0.148
Two viruses vs. four viruses	-49.833	21.866	-2.279	0.023	0.136
One virus vs. three viruses	-18.065	10.617	-1.702	0.089	0.533
One virus vs. four viruses	-42.148	21.436	-1.966	0.049	0.296
Three viruses vs. four viruses	-24.083	23.150	-1.040	0.298	1.000

The overall Kruskal–Wallis test demonstrated a significant difference in PICU length of stay across the four viral-burden groups (H=9.018, df=3, p=0.029). Pairwise comparisons were subsequently performed with Bonferroni adjustment for multiple testing. None of the pairwise comparisons remained statistically significant after adjustment. Asymptotic two-sided p values are reported. Group sizes were 73, 35, 15, and 3 patients for the one-, two-, three-, and four-virus groups, respectively. PICU: Pediatric intensive care unit.